Biochimica et Biophysica Acta 1813 (2011) 1845–1853

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Tau inhibits tubulin oligomerization induced by prion protein

Katarzyna M. Osiecka, Hanna Nieznanska, Krzysztof J. Skowronek, Jolanta Jozwiak, Krzysztof Nieznanski *

Department of Biochemistry, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

ARTICLE INFO

Article history:
Received 3 March 2011
Received in revised form 24 June 2011
Accepted 28 June 2011
Available online 6 July 2011

Keywords:
Prion protein
Tubulin
Tau
Phosphorylation
Prion disease
Alzheimer disease

ABSTRACT

In previous studies we have demonstrated that prion protein (PrP) interacts with tubulin and disrupts microtubular cytoskeleton by inducing tubulin oligomerization. These observations may explain the molecular mechanism of toxicity of cytoplasmic PrP in transmissible spongiform encephalopathies (TSEs). Here, we check whether microtubule associated proteins (MAPs) that regulate microtubule stability, influence the PrP-induced oligomerization of tubulin. We show that tubulin preparations depleted of MAPs are more prone to oligomerization by PrP than those containing traces of MAPs. Tau protein, a major neuronal member of the MAPs family, reduces the effect of PrP. Importantly, phosphorylation of Tau abolishes its ability to affect the PrP-induced oligomerization of tubulin. We propose that the binding of Tau stabilizes tubulin in a conformation less susceptible to oligomerization by PrP. Since elevated phosphorylation of Tau leading to a loss of its function is observed in Alzheimer disease and related tauopathies, our results point at a possible molecular link between these neurodegenerative disorders and TSEs.

1. Introduction

Cellular prion protein (PrP^C), particularly in a misfolded form (PrP^{Sc}), is widely considered a key player in the pathogenesis of neurodegenerative diseases called TSEs [1,2]. Nonetheless, the molecular mechanisms of the pathogenic misfolding as well as the PrP-mediated neurodegeneration remain unresolved. PrP^C is a mostly extracellular glycoprotein anchored in the plasma membrane [3]. In pathology, however, its intracellular concentration significantly raises. PrP point mutations linked to some TSEs increase the fraction of a transmembrane form of PrP (^{Ctm}PrP) with the C-terminal domain localized in endoplasmic reticulum (ER) lumen and the N-terminal domain exposed to the cytosol [4,5]. C-terminally truncated stop mutants of PrP are detected in the cytosol and nucleus [6,7]. Furthermore, accumulation of cytosolic PrP (cytoPrP) may result from the ER stress common in TSEs, affected translocation of nascent PrP, and direct inhibition of the proteasome by PrP^{Sc} [8–10]. Importantly, intracellular PrP has been demonstrated to be neurotoxic [4,11,12]. It is postulated that mislocalized PrP may exert toxic effects through interactions with intracellular proteins leading to loss or modification of their physiological functions [10,13]. In line with this hypothesis co-aggregation of several cytosolic proteins with cytoPrP has been reported. For example, cytoPrP co-aggregated the anti-apoptotic protein Bcl-2 and the ubiquitin ligase mahogunin [14,15]. Moreover, we have demonstrated that PrP binds to tubulin and disrupts microtubular cytoskeleton by inducing tubulin oligomerization and formation of aggregates [16–18]. Subsequently, it was also found that PrP^{Sc} co-immunoprecipitated with tubulin in brain homogenates of TSE-infected animals [19]. Interestingly, loss of dendritic microtubules has been observed in ultrastructural studies of TSE brain sections [20].

Tubulin is the major building block of microtubules – dynamic cytoskeletal structures involved in crucial cellular functions [21]. The assembly and stability of microtubules are regulated by microtubule associated proteins (MAPs) [22]. One of the major neuronal MAPs is Tau protein whose dysfunction leads to neurodegeneration observed in Alzheimer disease (AD) and other tauopathies [23]. Phosphorylation of Tau reduces its binding to tubulin and thereby regulates microtubule dynamics. Hyperphosphorylation of Tau, a hallmark of AD, leads to its aggregation into paired helical filaments, disassembly of microtubular cytoskeleton, and neurodegeneration. Interestingly, hyperphosphorylation of Tau has also been reported in human TSEs cases [24–27]. This may result from induction of Tau hyperphosphorylation by misfolded PrP [28,29] indicating a molecular link between these protein misfolding diseases (PMDs). Furthermore, a direct interaction between PrP and Tau has been demonstrated [30].

Here, we checked whether MAPs/Tau protein may influence the PrP-induced oligomerization of tubulin. We have also verified an intriguing scenario where phosphorylation of Tau enhances the

Abbreviations: AD, Alzheimer disease; CBB, Coomassie brilliant blue; ^{Ctm}PrP, transmembrane form of prion protein with the C-terminus residing in the lumen of endoplasmic reticulum; cytoPrP, cytosolic prion protein; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; ER, endoplasmic reticulum; GPI, glycosylphosphatidylinositol; GSK3, glycogen synthase kinase 3; mAb, monoclonal antibody; MAPs, microtubule-associated proteins; pep1-30, peptide corresponding to PrP sequence 1–30; PKA, protein kinase A; PMDs, protein misfolding diseases; PrP, prion protein; PrP^C, cellular form of prion protein; PrP^{Sc}, TSE (scrapie) form of prion protein; rTau, recombinant Tau; SNHS, *N*-hydroxysulfosuccinimide; TSEs, transmissible spongiform encephalopathies

* Corresponding author. Tel.: +48 22 5892318; fax: +48 22 8225342.
E-mail address: k.nieznanski@nencki.gov.pl (K. Nieznanski).

0167-4889/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2011.06.016

deleterious effect of PrP exerted at the level of the microtubular cytoskeleton.

2. Materials and methods

2.1. Protein purification

Human prion protein (residues 23–231) was expressed in *E. coli* and purified as described previously [31,32]. As it was demonstrated by Zahn and colleagues [32], the applied purification procedure gives natively folded PrP. To ensure that PrP was not aggregated the preparations were ultracentrifuged (30 min at 200,000 *g*, 30 °C) before each experiment.

Tubulin containing traces of MAPs (crude tubulin) was purified from porcine brain by two cycles of polymerization/depolymerization according to the modified method of Mandelkow and colleagues [33], described in details in [16]. The last step of microtubule depolymerization was performed either for 2 or 12 h. Tubulin preparations were stored at −70 °C in PM buffer composed of 2 mM EGTA, 0.1 mM EDTA, 1 mM ATP, 2 mM DTT and 100 mM PIPES pH 6.9. Just before experiments the preparations were thawed and centrifuged for 20 min at 22,000 *g*, 4 °C. Obtained supernatants were used as crude tubulin.

Crude tubulin was depleted of MAPs by the modified method of Castoldi and Popov [34]. Tubulin supernatant, obtained as described above, was incubated with equal volume of 20% DMSO, 2 mM $MgCl_2$, 2 mM GTP and 1 M PIPES pH 6.9 for 1 h at 37 °C. The mixture was then layered (1:2, v/v) over a cushion composed of 60% glycerol, 1 mM EGTA, 1 mM $MgCl_2$, 80 mM PIPES pH 6.9 (warmed to 37 °C), and centrifuged for 1 h at 200,000 *g*, 37 °C. The obtained pellet of pure microtubules was briefly rinsed and homogenized in PM buffer on ice. The resuspended pellet was used at the day of preparation as pure tubulin. The supernatant, was dialyzed against 0.3 M KCl, 1 mM EDTA, 0.1 mM DTT, 0.5 mM PMSF and 10 mM MOPS pH 6.8 and subsequently used for preparation of MAPs fraction. MAPs were separated from remnants of tubulin on DEAE Sephacel according to Murphy and colleagues [35]. The fractions containing MAPs were concentrated on Centriprep (Millipore, Billerica, MA, USA) and dialyzed against 50 mM NaCl, 1 mM EDTA, 0.1 mM DTT, 0.5 mM PMSF and 10 mM MOPS pH 6.8, and stored at −70 °C.

Brain Tau was purified directly from crude tubulin or from supernatants obtained upon centrifugation of crude tubulin over the glycerol cushion. Tau preparations of different electrophoretic mobility were obtained from tubulin depolymerized by incubation for different lengths of time (see above). Generally, preparations of higher electrophoretic mobility (H) were obtained from tubulin depolymerized for 2 h whereas those of lower mobility (L) from tubulin depolymerized for 12 h. Initial preparations were dialyzed against 0.75 M NaCl, 1 mM $MgCl_2$, 2 mM EGTA, 2% β-ME, 0.5 mM PMSF and 20 mM MES pH 6.8. Then, according to the method of Fellous and colleagues [36], the preparations were boiled for 5 min in a water bath and after cooling centrifuged for 30 min at 29,000 *g*, 4 °C. Obtained supernatants were treated with 2.5% perchloric acid as described by Lindwall and Cole [37], and immediately centrifuged for 15 min at 14,000 *g*, 25 °C. Subsequently, Tau was salted out from supernatants by ammonium sulfate at 50% saturation. The preparation was centrifuged for 30 min at 42,000 *g*, 4 °C. Tau pellet was resuspended, dialyzed against 50 mM NaCl, 0.5 mM EGTA, 1 mM DTT, 0.5 mM PMSF and 50 mM MES pH 6.8, and stored at −70 °C.

Recombinant Tau (rTau, human isoform 2N4R) was purchased from Sigma (St. Louis, MO, USA). If not indicated otherwise, rTau at 28 μg/ml (0.6 μM) was used.

2.2. Phosphorylation of Tau

rTau at 0.85 mg/ml was incubated with catalytic subunit of PKA (Sigma) added at the proportion of 60 μg/mg of Tau for 2 h at 37 °C in 28 mM NaCl, 10 mM $MgCl_2$, 0.28 mM EGTA, 1.2 mM DTT and 28 mM MES pH 6.8. The phosphorylation was initiated by addition of 1 mM ATP. After 2 h, the buffer was supplemented with 0.01% Triton X-100 and the concentrations of NaCl, DTT and ATP were increased to 76 mM, 1.8 mM and 2 mM, respectively, whereas pH was increased to 7.4 with 16 mM Tris–HCl. The mixture was then incubated with GSK3β (GenScript, Piscataway, NJ, USA) at the proportion of 26 μg/mg of Tau for 3 h at 37 °C. In some experiments Tau was phosphorylated solely by PKA for 5 h. Solubility of phosphorylated Tau was confirmed in sedimentation experiments by centrifugation for 10 min at 200,000 *g*, 4 °C. The extent of phosphorylation was assessed by shift in electrophoretical mobility upon SDS-PAGE.

2.3. Dephosphorylation of Tau

Brain Tau at 0.4 mg/ml was incubated with phosphatase PP2A (Millipore) at the proportion of 17 μg/mg of Tau for 2 h at 30 °C in 240 mM NaCl, 0.4 mM $MgCl_2$, 0.04 mM $MnCl_2$, 0.7 mM EGTA, 25 mM β-ME, 1 mM DTT, 0.3 mM PMSF and 30 mM MES pH 6.8. The extent of dephosphorylation was assessed by SDS-PAGE and Western blotting.

2.4. Light scattering

Oligomerization of tubulin was assessed by turbidity measurements as previously described [17] at conditions not allowing microtubule formation. Tubulin at 0.2 mg/ml (4 μM, assuming molecular weight ≈ 50,000) was incubated at 25 °C in a buffer containing 10% (w/v) glycerol, 1 mM GTP, 16 mM $MgCl_2$ and 10 mM sodium phosphate buffer pH 7.0. In some experiments the buffer was supplemented with 50 μM $CaCl_2$. Monitoring of turbidity was initiated immediately after addition of PrP (final concentration of 10 or 15 μg/ml, corresponding to 0.4 or 0.6 μM) to the cuvette. All measurements were performed against tubulin or tubulin with MAPs/Tau as a reference.

2.5. Cross-linking experiments

Covalent cross-linking was performed at the same conditions and protein concentrations as the turbidity measurements. The reaction was initiated by addition of 1 mM SNHS and 1 mM EDC. After 1 h of incubation at 25 °C, cross-linking was stopped with 50 mM Tris. The cross-linking products were analyzed by SDS-PAGE and Western blotting.

2.6. Transmission electron microscopy

The samples analyzed by light scattering were also subjected to electron microscopic studies. After 20 min of incubation at 25 °C, 10-μl samples were placed on copper grids (400 mesh, Sigma) covered with collodion (SPI Supplies, West Chester, PA, USA) and carbon. Negative staining was performed with 2% (w/v) uranyl acetate (SPI Supplies) for 25 s. The grids were examined in a JEM 1400 electron microscope (JEOL Co., Japan) equipped with a digital camera (CCD MORADA, SiS-Olympus, Germany).

2.7. Electrophoretic separations

SDS-PAGE was performed on 10% separating gels according to Laemmli [38]. To improve separation of Tau preparations, the electrophoresis was continued until the 50 kDa marker reached the edge of the gel. The gels were stained with Coomassie brilliant blue R-250.

2.8. Western blotting

After SDS-PAGE proteins were electrotransferred onto nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ, USA) in a standard

transfer buffer supplemented with 0.05% SDS. PrP was detected with a primary antibody mAb 6H4 (Prionics AG, Schlieren, Switzerland) diluted at 1:5,000. Tau was detected by primary antibody mAb T8201 (Sigma) diluted at 1:1,000. Then, goat secondary antibody AP308P anti-mouse IgG (Millipore) conjugated with horseradish peroxidase (HRP) diluted at 1:5,000 was used. β-actin was detected by antibody mAb A3854 (Sigma) conjugated with HRP, diluted at 1:100,000. Antigen detections were carried out by incubation of the blots with a chemiluminescent HRP substrate (Millipore) followed by exposition against X-ray films (Kodak, Rochester, NY, USA).

2.9. Peptide synthesis

Peptide MANLGYWLLALFVTMWTDVGLCKKRPKPGG-NH_2 (pep1-30) corresponding to mouse PrP sequence 1–30 (residues 23–30 identical to human PrP), was synthesized commercially (EZBiolab, Westfield, IN, USA). The peptide was of 96% purity as determined by HPLC and mass spectrometry analyses. Pep1-30 was dissolved in deionized water.

2.10. Cell culture

Human epithelial cells (HEp-2) were purchased from American Type Culture Collection (Rockville, MD, USA) and were cultured in modified Eagle's medium containing 25 mM glucose, 2 mM L-glutamine, 10% fetal bovine serum, 100 units/ml penicillin and, 100 μg/ml streptomycin. Cells were grown in a humidified incubator with 5% CO_2 at 37 °C. The culture medium was changed every 2–3 days and cells were passaged when confluent.

Plasmid pET29b encoding human Tau (2N4R) described by Hedgepeth and colleagues [39] was purchased from Addgene (Cambridge, MA, USA). Tau coding sequence was amplified from plasmid Tau/pET29b with primers tau5Not (5′-TAGCGGCCG-CATGGCTGAGCCCCGCCAG-3′) and tauSTOP3Bgl2 (5′-TAAGATCTAT-CACAAACCCTGCTTGGCCAGG-3′). PCR product was digested with NotI and BglII and cloned into p3xFLAG-CMV-14 vector (Sigma) digested with the same enzymes. Correctness of the final construct was confirmed by dideoxy sequencing. The cells were transfected either with Tau/pCMV14 or empty plasmid using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

Transfected HEp-2 cells seeded the day before experiment onto coverslips were treated with 0.5 or 1 μM (final concentration) pep1-30 in the culture medium. After 1 h the cells were fixed for 20 min with 3% paraformaldehyde in PBS.

2.11. Immunofluorescence microscopy

Fixed HEp-2 cells were washed with PBS and treated with 50 mM NH_4Cl in PBS for 15 min, washed with PBS and permeabilized with 0.05% Triton X-100 in PBS for 4 min at 4 °C. After washing with PBS the cells were incubated with 2% horse serum in PBS for 1 h at 25 °C and subsequently incubated with antibodies to β-tubulin (T0198, Sigma) diluted at 1:500 in 2% horse serum (in PBS) for 16 h at 4 °C. The cells were then washed with PBS and incubated with antibodies to mouse IgG conjugated with ALEXA 546 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) at 1:1000 for 1 h at room temperature, washed with PBS and mounted on glass slides. The cells were analyzed in confocal microscope Leica TCS SP5.

3. Results

Previously we described the effects of PrP on tubulin preparations containing traces of endogenous MAPs (crude tubulin). Those studies showed that PrP induced rapid oligomerization and aggregation of tubulin [17,18]. Since MAPs regulate the stability of structures formed by tubulin, here we checked the influence of these proteins on the PrP effect on tubulin oligomerization. First we compared the effects of PrP on crude tubulin (containing traces of MAPs) and tubulin depleted of MAPs (pure tubulin). Co-purified MAPs constituted ~10% of total protein in crude tubulin preparations. We conducted these studies at conditions not allowing microtubule formation (low tubulin concentration) to avoid co-occurrence of tubulin oligomerization by PrP with microtubule assembly by MAPs, both resulting in an increase in the turbidity of tubulin samples. As previously reported [17] tubulin alone gives no light scattering. Fig. 1A shows that the turbidity induced by PrP in preparations of tubulin containing endogenous MAPs is much weaker than that in preparations of pure tubulin. This suggests that crude tubulin is less susceptible to PrP-induced oligomerization than is pure tubulin. As expected, MAPs fraction isolated from preparations of crude tubulin reduced the PrP-induced oligomerization of pure tubulin (Fig. 1B). The MAPs fraction added alone to tubulin induced no light scattering (data not shown) indicating that in fact microtubules were not formed at the conditions applied. Since Tau protein is one of the major components of neuronal MAPs fraction we checked whether it was also present in our preparations. As the Western blot (inset in Fig. 1A) shows, crude tubulin contained Tau whereas tubulin depleted of MAPs was free of this protein. Tau was also present in the isolated MAPs fraction used in experiments demonstrated in Fig. 1B (see the CBB-stained gel and Western blot in Fig. 1B).

To check whether Tau is responsible for the effect described above we purified it from brain tubulin preparations. Tau preparations of different electrophoretic mobility were obtained (Western blot in Fig. 2, compare lanes H and L), depending on the tubulin purification

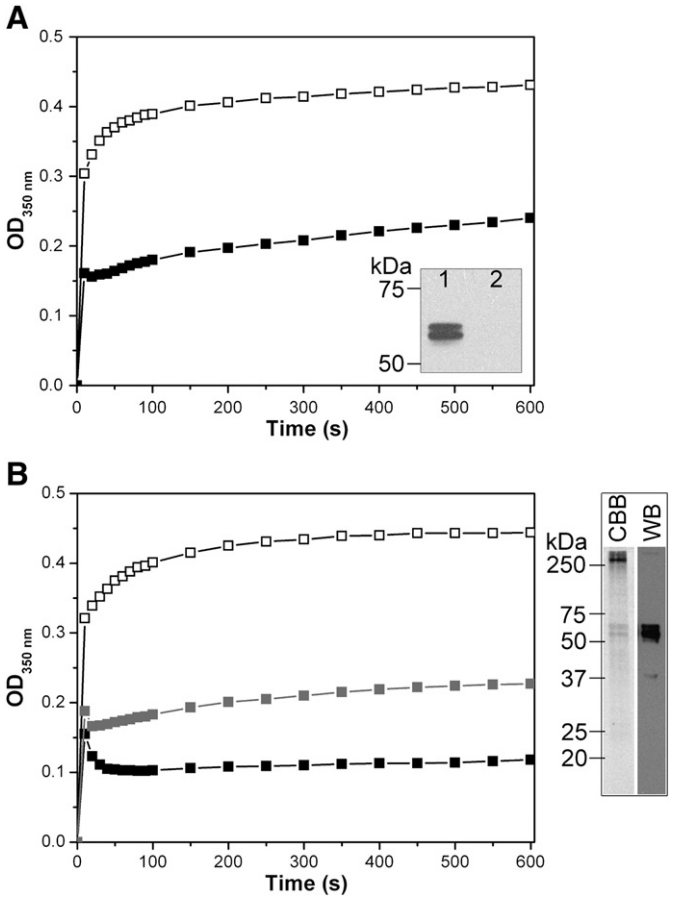

Fig. 1. MAPs reduce PrP-induced turbidity of tubulin. In A tubulin (0.2 mg/ml) containing endogenous MAPs (■) or tubulin depleted of MAPs (□) was incubated with PrP (10 μg/ml). Inset shows Tau detected by Western blotting in preparation of tubulin containing endogenous MAPs (1) but not in pure tubulin preparation (2). In B tubulin (0.2 mg/ml) depleted of MAPs was incubated with PrP (10 μg/ml) in the presence of isolated MAPs fraction at 0 (□), 40 (▩) or 80 μg/ml (■). Inset shows MAPs fraction on Coomassie-stained gel (CBB) and Western blot analyzed with anti-Tau antibody (WB).

procedure used (see Section 2.1). Addition of a Tau fraction of higher electrophoretic mobility (H) to pure tubulin resulted in the reduction of PrP-induced oligomerization (Fig. 2A), whereas a Tau fraction of lower electrophoretic mobility (L) had no effect on the oligomerization (Fig. 2B). Either Tau preparation added alone did not influence the light scattering by tubulin samples (data not shown). The observed differences in the apparent molecular mass of Tau may result from its varied level of phosphorylation, the major posttranslational modification of Tau affecting its interaction with tubulin. Therefore we checked whether phosphorylation was in fact responsible for the different features of our Tau preparations. We subjected the Tau fraction of lower electrophoretic mobility (L), which was ineffective in inhibition of the PrP-induced tubulin oligomerization (Fig. 2B), to dephosphorylation by phosphatase PP2A. Treatment with PP2A increased the electrophoretic mobility of Tau (Western blot in Fig. 3, compare lanes marked "−" and "+") accompanied by a substantial restoration of the ability to decrease the PrP-induced oligomerization of tubulin (Fig. 3). Subsequently, we showed that recombinant human Tau (rTau) purified from *E. coli* inhibited the PrP-induced tubulin oligomerization (Fig. 4A), indicating that this effect requires no eukaryotic posttranslational modifications.

It is widely accepted that GSK3 is the main kinase responsible for modification of Tau at physiological as well as pathological conditions

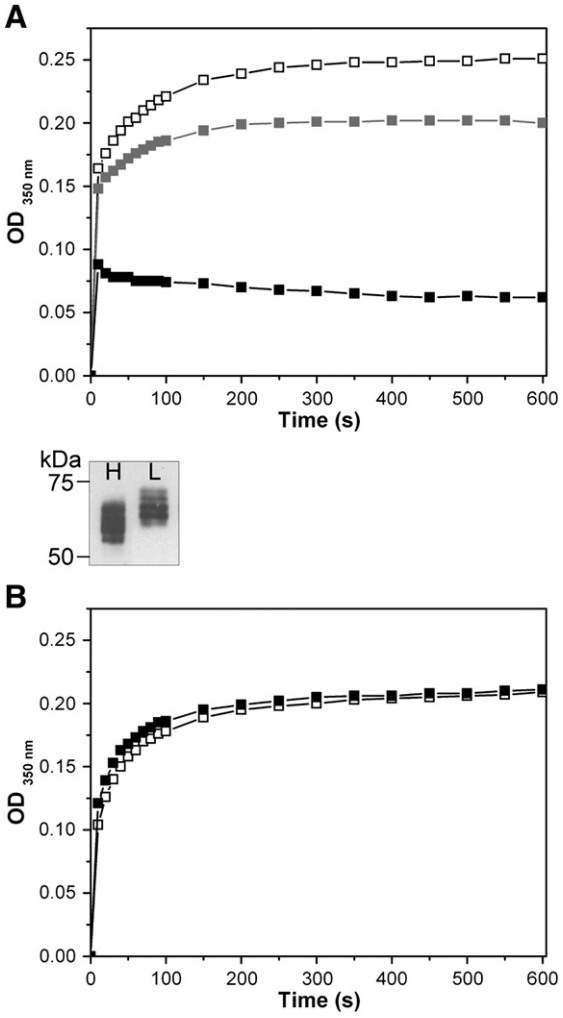

Fig. 2. Effect of Tau purified from brain on PrP-induced turbidity of tubulin. In A pure tubulin (0.2 mg/ml) was incubated with PrP (15 μg/ml) in the presence of 0 (□), 28 (▩) or 56 μg/ml (■) of Tau fraction of high electrophoretic mobility (H). In B the incubation was performed in the absence (□) or presence (■) of Tau fraction of lower electrophoretic mobility (L, 56 μg/ml). Inset shows Western blot for Tau fractions of higher (H) and lower electrophoretic mobility (L).

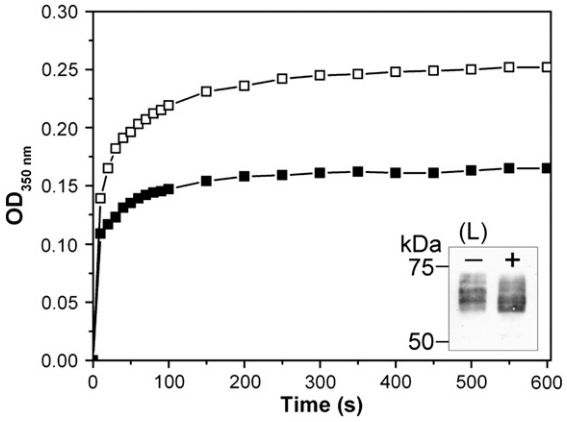

Fig. 3. Dephosphorylated brain Tau regains ability to reduce PrP-induced turbidity of tubulin. Tubulin (0.2 mg/ml) was incubated with PrP (15 μg/ml) in the absence (□) or presence (■) of Tau fraction of lower electrophoretic mobility (L, 56 μg/ml) subjected to dephosphorylation with phosphatase PP2A. Compare with Fig. 2B obtained for L fraction of Tau before dephosphorylation. Western blot shows untreated (−) and dephosphorylated with PP2A (+) Tau.

(rev. in [23]). It has also been demonstrated that phosphorylation of Tau by GSK3 is enhanced by prior phosphorylation by PKA [40]. Hence, to confirm the role of phosphorylation, we subjected purified recombinant Tau to sequential modification by PKA and GSK3. As it is shown in Fig. 4, unphosphorylated rTau reduces the PrP-induced oligomerization (Fig. 4A) whereas the phosphorylated protein is ineffective (Fig. 4B). As expected, phosphorylated rTau exhibited a decreased electrophoretic mobility (gel in Fig. 4B, compare lanes 1 and 2). Similar effect of phosphorylation by PKA/GSK3 we have observed for dephosphorylated brain Tau (see Supplementary data, Fig. 1S). Interestingly, phosphorylation by PKA alone had no effect on the ability of rTau to reduce the PrP-induced tubulin oligomerization (Fig. 4C), although Tau was readily modified by this kinase (gel in Fig. 4C, compare lanes 1 and 2). Neither phosphorylated nor untreated rTau added alone increased the light scattering by tubulin samples (data not shown).

We then compared the ability of unmodified rTau and rTau phosphorylated by PKA/GSK3 to bind to tubulin. As reported by Sengupta and colleagues [41], the protein phosphorylated by both kinases exhibited reduced affinity to microtubules, whereas that modified by PKA alone had an almost unchanged affinity. Consistently, reduced binding to tubulin was also demonstrated for rTau phosphorylated with PKA/GSK3 in our cross-linking experiments performed at the conditions not allowing microtubule formation (Fig. 5). These observations suggest that the phosphorylation may lessen the influence of Tau on the PrP-induced oligomerization by reducing the binding of Tau to tubulin.

To dissect the molecular mechanism of Tau action further we examined the effects at the level of PrP interaction with tubulin. We made use of the observation that PKA/GSK3-phosphorylated Tau lacks the ability to affect the PrP-induced oligomerization of tubulin (Fig. 4B). We compared the binding of PrP to tubulin in the absence or presence of unphosphorylated rTau or rTau phosphorylated by PKA/GSK3 under conditions of the light scattering experiments. As demonstrated in Fig. 6, the yield of PrP-tubulin cross-linking is not affected by Tau protein, independently of its phosphorylation status. This suggests that Tau does not exert its effect by affecting the binding of PrP to tubulin.

Since calcium ions compete with Tau for a binding site in the C-terminal domain of tubulin [42], we checked whether this cation could also abolish the Tau effect on the PrP-induced tubulin oligomerization. As demonstrated in Fig. 7, Ca^{2+} does not affect the PrP-induced oligomerization, implying that the binding sites on

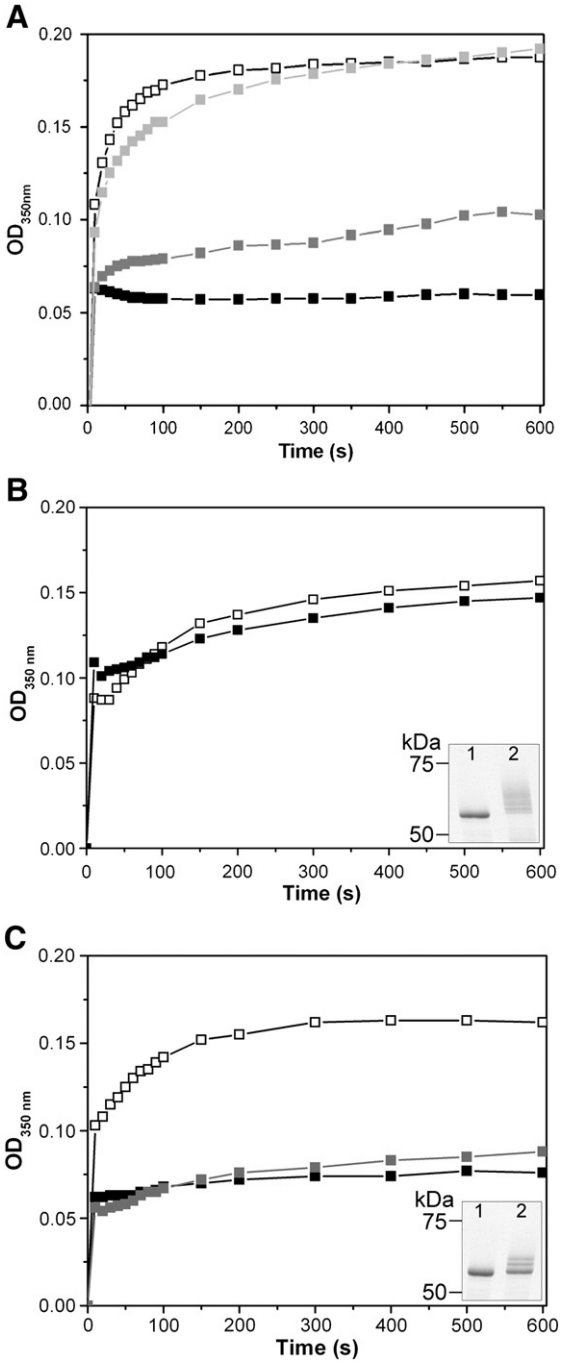

Fig. 4. A. Recombinant Tau reduces PrP-induced turbidity of tubulin. Tubulin (0.2 mg/ml) was incubated with PrP (15 μg/ml) in the absence (□) or presence of rTau at 7 (▒), 14 (▓) or 28 μg/ml (■). The tubulin/PrP/rTau molar ratios were 4:0.6:0.15 or 0.3 or 0.6, respectively. Note barely detectable effect of rTau (during first two min of assay) even at as low as 0.15:4 molar ratio to tubulin. B. Sequential phosphorylation of recombinant Tau by PKA and GSK3 kinases abolishes its effect on PrP-induced turbidity of tubulin. Tubulin (0.2 mg/ml) was incubated with PrP (15 μg/ml) in the absence (□) or presence (■) of rTau (28 μg/ml) phosphorylated sequentially with PKA and GSK3. The tubulin/PrP/rTau molar ratio was 4:0.6:0.6. Inset shows Coomassie-stained gel for rTau untreated (1) and phosphorylated with PKA/GSK3 (2). C. Phosphorylation of recombinant Tau by PKA alone has no effect on PrP-induced turbidity of tubulin. Tubulin (0.2 mg/ml) was incubated with PrP (15 μg/ml) in the absence (□) or presence of untreated rTau (28 μg/ml, ■) or rTau (28 μg/ml) phosphorylated with PKA (▒). The tubulin/PrP/rTau molar ratio was 4:0.6:0.6. Coomassie-stained gel shows rTau untreated (1) and phosphorylated with PKA alone (2).

tubulin for Tau and Ca^{2+} are, at least partially, different from that for PrP. Importantly, calcium significantly reduced the Tau effect on the PrP-induced oligomerization (Fig. 7). This suggests that Tau influences

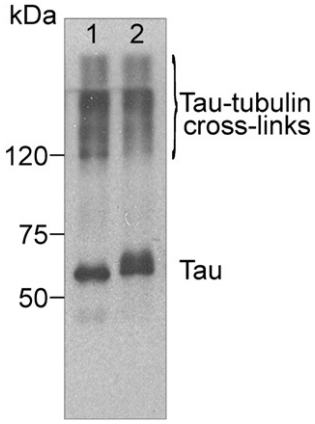

Fig. 5. Phosphorylation of Tau reduces its binding to tubulin. Tubulin (0.2 mg/ml) was cross-linked by EDC with untreated (1) or phosphorylated with PKA/GSK3 (2) rTau (28 μg/ml). The tubulin/rTau molar ratio was 4:0.6. Western blot was analyzed with anti-Tau antibody. Bands migrating above 120 kDa marker correspond to cross-linking products of Tau with tubulin monomers (dissociated by SDS), tubulin heterodimers and higher tubulin oligomers. Note reduced concentration of the cross-links with phosphorylated tau.

the PrP-induced oligomerization by interaction with tubulin in a region different from the docking site for PrP.

To gain an insight into the effect of Tau on the structures formed by tubulin, we performed electron microscopic analysis. In these studies we compared the morphology of tubulin samples in the presence of PrP alone or PrP together with rTau (Fig. 8). As formerly demonstrated [17,18], PrP induced tubulin oligomerization and assembly of large aggregates of tubulin oligomers. Also numerous ring-shaped structures and their fragments, both characteristic for tubulin incubated at conditions not allowing microtubule formation and at high concentrations of magnesium [43,44], were observed in these samples (Fig. 8). As expected, oligomerization of tubulin and formation of aggregates was inhibited in samples containing Tau (Fig. 8). This was accompanied by an increase in the number of the ring-shaped structures. The number of small globular oligomers found in tubulin incubated alone was reduced after addition of Tau. These oligomers were almost absent in the presence of both Tau and PrP. Notably, we found no ring-shaped structures in tubulin incubated alone, whereas they were present in samples containing Tau or PrP and were most abundant in the presence of Tau together with PrP. These observations

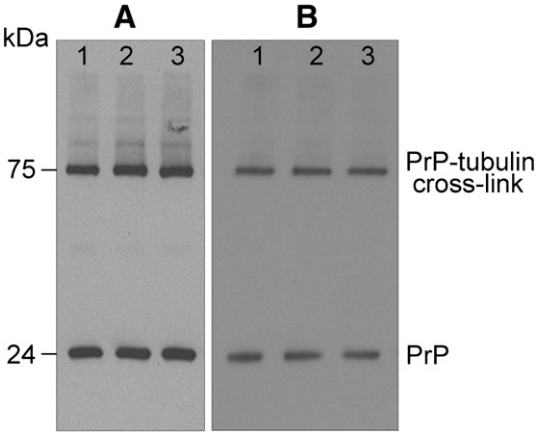

Fig. 6. Tau does not reduce PrP binding to tubulin. In A PrP (15 μg/ml) was cross-linked by EDC with tubulin (0.2 mg/ml) in the absence (1) or presence of untreated (2) or dephosphorylated with PP2A (3) brain Tau (L, 56 μg/ml). In B PrP (15 μg/ml) was cross-linked with tubulin (0.2 mg/ml) in the absence (1) or presence of untreated (2) or phosphorylated with PKA/GSK3 (3) rTau (28 μg/ml). The tubulin/PrP/rTau molar ratio was 4:0.6:0.6. Western blots were analyzed with anti-PrP antibody.

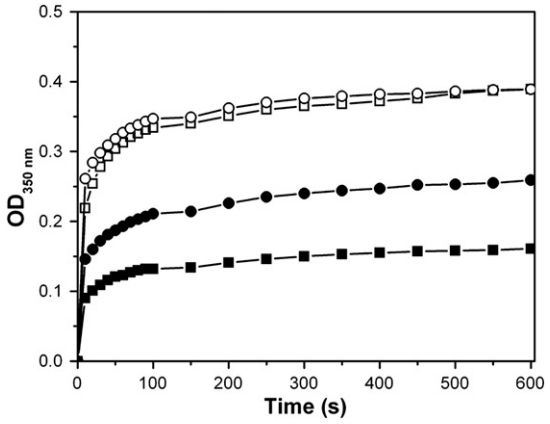

Fig. 7. Calcium reduces effect of Tau on PrP-induced turbidity of tubulin. Tubulin (0.2 mg/ml) was incubated with PrP (15 μg/ml) in the absence (□) or presence (■) of rTau (28 μg/ml). After addition of calcium, tubulin was similarly incubated with PrP (15 μg/ml) in the absence (○) or presence (●) of rTau (28 μg/ml). The tubulin/PrP/rTau molar ratio was 4:0.6:0.6.

suggest that Tau stabilizes tubulin in the form of ring-shaped oligomers (or other amorphous structures) which are less susceptible to PrP-mediated oligomerization and aggregation.

To check whether Tau is capable of protecting the microtubular cytoskeleton against PrP-induced disassembly, we subjected human epithelial cells (HEp-2) transfected either with empty or Tau-encoding plasmid to treatment with PrP peptide 1–30 (pep1-30). This peptide encompasses the major tubulin binding site (PrP residues 23–30) responsible for tubulin oligomerization and inhibition of microtubule assembly [18]. As it was demonstrated previously, pep1-30 of known ability to penetrate into cytoplasm of cultured cells [45], induced disruption of the microtubular cytoskeleton of HEp-2 cells [18]. Also in this study, already 0.5 μM pep1-30 caused disintegration of the microtubular cytoskeleton accompanied by rounding of the cells lacking Tau protein (Fig. 9C, E). At the same time, the cells transfected with plasmid encoding Tau were apparently insusceptible to the peptide applied at 0.5 μM concentration (Fig. 9D) and much more resistant to 1 μM peptide than the cells transfected with empty plasmid (Fig. 9F).

4. Disussion

In this study we have found that Tau protein may substantially decrease the PrP-induced oligomerization of tubulin. As we have demonstrated previously this oligomerization leads to inhibition of microtubule formation which may have adverse consequences to the cell [17]. Employing a membrane-penetrating peptide we have shown that PrP fragment 1–30 encompassing the major tubulin binding site (PrP residues 23–30) disrupts microtubular cytoskeleton of cultured cells [18]. That observation was further confirmed in cells transfected with a plasmid encoding cytoPrP [46]. Herein, we have found that Tau protects cultured cells from deleterious effect of PrP peptide 1–30. In light of our present results one can assume that Tau is a negative

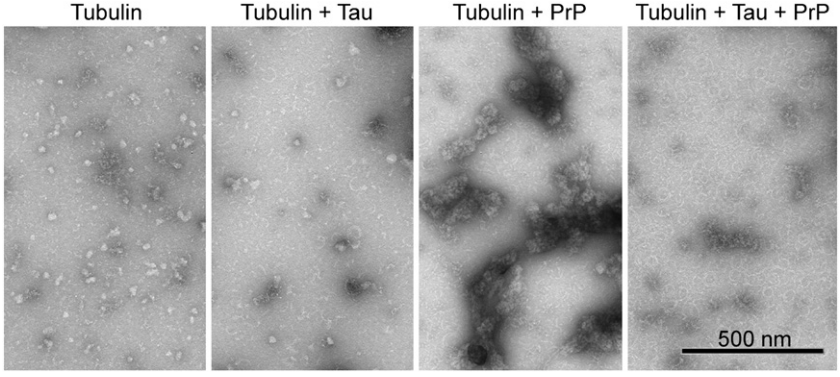

Fig. 8. Tau inhibits formation of PrP-induced tubulin oligomers/aggregates. Electron microscopy of tubulin (0.2 mg/ml) alone, tubulin incubated with PrP (15 μg/ml), tubulin incubated with rTau (28 μg/ml) or tubulin incubated with both rTau (28 μg/ml) and PrP (15 μg/ml). The tubulin/PrP/rTau molar ratio was 4:0.6:0.6.

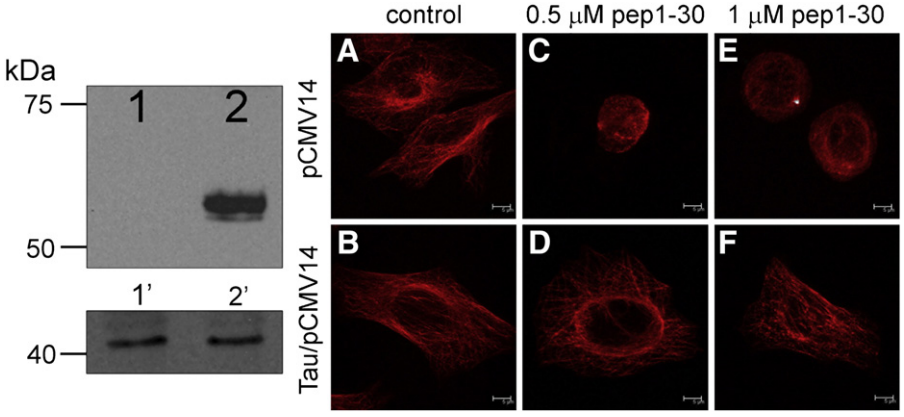

Fig. 9. Tau protects microtubular cytoskeleton of cultured cells from disassembly by PrP peptide 1–30. Western blot shows that HEp-2 cells transfected with Tau-encoding plasmid (lane 2) but not these transfected with empty plasmid (lane 1) express Tau protein. β-actin is shown as a loading control (lanes 1′ and 2′). Confocal fluorescence microscopy of untreated HEp-2 cells (A, B) as well as treated with 0.5 (C, D) or 1 μM pep1-30 (E, F) was performed using antibodies to β-tubulin to visualize microtubular cytoskeleton. Note that the microtubular cytoskeleton of peptide-treated cells transfected with empty vector (pCMV14) is disassembled and tubulin is congregated around the nuclei that is accompanied by cell rounding (C, E). At the same time, cells transfected with plasmid encoding Tau (Tau/pCMV14) exhibited normal morphology with tubulin forming a network of linear structures in the entire cytoplasm in the presence of 0.5 μM pep1-30 (D). Tau-expressing cells seem to be more resistant than the control cells also to 1 μM peptide (F). Scale bar is 5 microns.

regulator of PrP-linked disassembly of microtubular cytoskeleton. Furthermore, we have found that phosphorylation of Tau that, at an elevated level, is a hallmark of AD, may indirectly enhance deleterious effect of PrP on tubulin function.

What is the mechanism of Tau action? The above-described inhibition of the PrP-induced tubulin oligomerization could in principle result from: (i) competition between Tau and PrP for a binding site on tubulin, (ii) interaction between Tau and PrP or (iii) Tau-mediated stabilization of a tubulin structure less susceptible to the oligomerization. Our observations imply that the Tau effect is mediated through its interaction with tubulin, but is not a result of competition between Tau and PrP for a binding site on tubulin. We have found that neither unphosphorylated nor PKA/GSK-modified Tau affects PrP-tubulin cross-linking. On the other hand, phosphorylation of Tau by PKA/GSK reduces its binding to tubulin [40] as well as its ability to prevent the PrP-induced oligomerization. Accordingly, phosphorylation by PKA alone, which does not reduce Tau binding to tubulin [41], also does not influence the Tau effect. Furthermore, in our experiments, calcium reduced the Tau effect on the PrP-induced oligomerization. This may be explained by the observation that calcium competes with Tau for binding to the C-terminal domain of tubulin [42]. Conversely, calcium did not affect the PrP-induced oligomerization of tubulin, indicating that the binding sites for this cation and PrP do not overlap. In fact, subtilisin-treated tubulin lacks high affinity calcium binding sites [47] whereas its ability to bind PrP is not affected [18]. Altogether, the Tau effect on the PrP-induced oligomerization is lessened when the binding of Tau to tubulin is reduced either by phosphorylation or by calcium ions.

We found that even traces of MAPs present in preparations of crude tubulin are capable of significantly reducing the PrP effect. This is consistent with the observation that traces of MAPs stabilize microtubules efficiently [48]. This also suggests that MAPs/Tau exert their effect through stabilization of particular structures of tubulin, e.g. ring-shaped oligomers rather than through hindering the PrP binding to tubulin. Since MAPs are only present at a low concentration in crude tubulin preparations, it is quite unlikely that they act through sequestration of PrP.

We suppose that all MAPs which are able to promote assembly and stabilize microtubules can also make tubulin less susceptible to oligomerization by PrP. Our preliminary studies indicate that also MAP2 is able to inhibit PrP-induced oligomerization of tubulin (unpublished observations). However, in this paper we focused on Tau since hyperphosphorylation of this MAP, leading to loss of its microtubule-stabilizing function, has been reported extensively in prion diseases. Furthermore, hyperphosphorylated Tau sequesters not only normal Tau but also other MAPs like MAP1 and MAP2 (rev. in [23]). Therefore, Tau seems to be the key MAP in the neurodegeneration processes related to microtubule disruption. Since Tau is localized mainly in axons whereas MAP2 in dendrites, the effects of cytosolic PrP on microtubular cytoskeleton could be controlled in both types of neurites. Phosphorylation of MAPs is a common mechanism regulating their interaction with tubulin hence it is reasonable to assume that

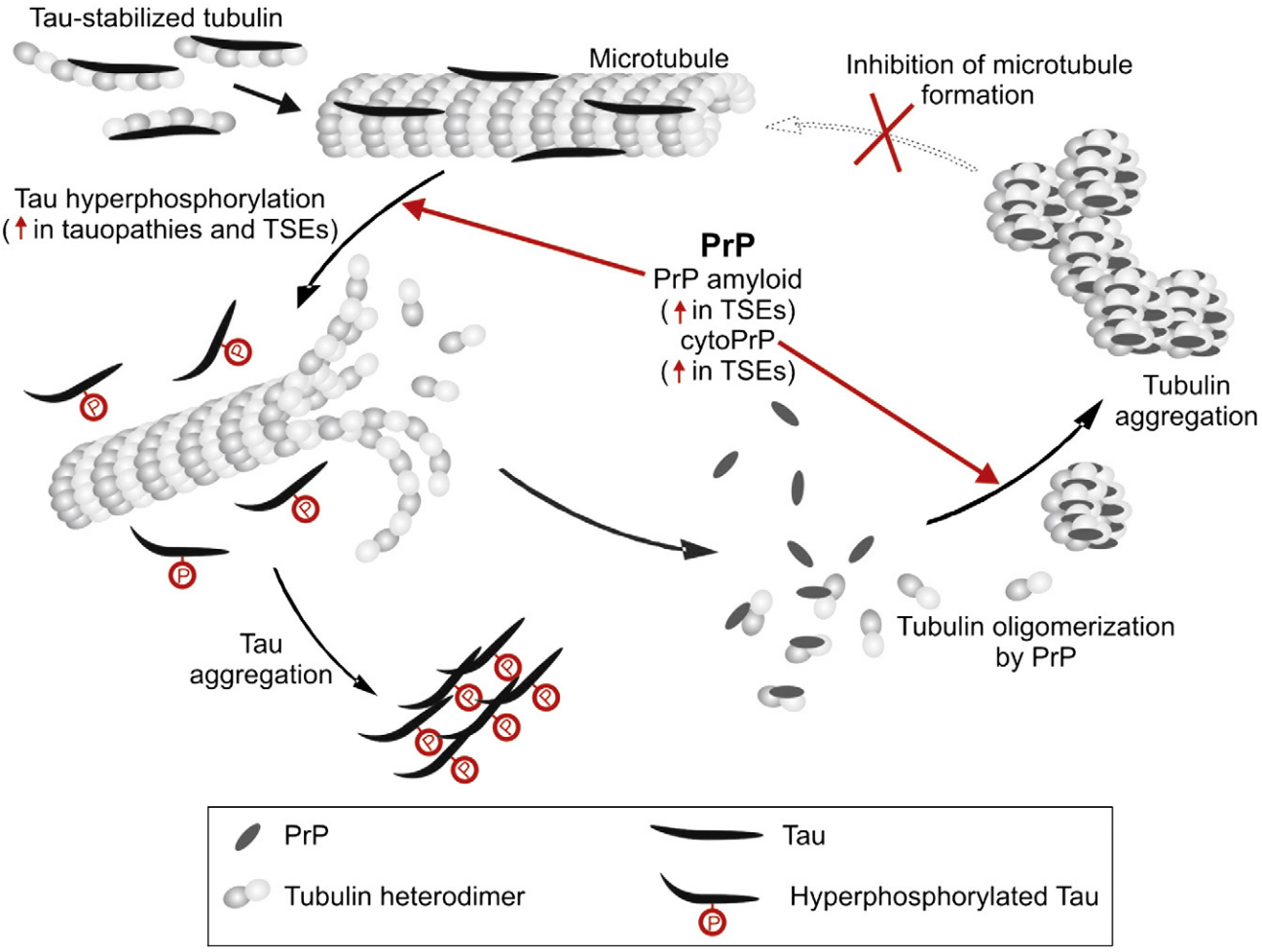

Fig. 10. Proposed molecular cross-talk between hyper-phosphorylation of Tau and toxicity of cytosolic PrP. In TSEs misfolded PrP induces phosphorylation of Tau. This modification leads to dissociation of Tau from tubulin and disassembly of microtubules. Tubulin depleted of Tau becomes more susceptible to oligomerization/aggregation by cytoPrP whose concentration is also elevated in TSEs. Tubulin co-aggregated with PrP is unable to form microtubules.

this modification may modulate effect of all MAPs on PrP-induced oligomerization.

In the light of our results, of particular relevance are observations suggesting that PrP may trigger phosphorylation of Tau. Accumulation of hyperphosphorylated Tau in brains of TSE patients is amply documented [24–27]. Deposits of hyperphosphorylated Tau, indistinguishable from those observed in AD, are frequently found in vicinity of PrP plaques. Plaques with cores composed of PrP aggregates, surrounded by Tau deposits, have been reported [49,50]. Hyperphosphorylation of Tau has also been observed in animals experimentally infected with TSE [51,52]. This co-occurrence of the hallmarks of TSE and AD may have important pathological implications. The spatial proximity of PrP and Tau aggregates suggests that they influence each other's formation and/or cytotoxic effects. In fact, it has been demonstrated that cells treated with amyloid composed of PrP fragment 106–126 exhibit increased activity of GSK3 and cdk5 accompanied by hyperphosphorylation of Tau [28,29]. Another molecular link between TSEs and AD has been provided by the observation of PrP deposits in brains of patients with a familial form of AD [53]. Recently, induction of Aβ peptide aggregation – which is also a hallmark of AD – by PrP^{Sc} as well as aggregation of PrP by amyloid of Aβ has been demonstrated [54]. These observations indicate that pathology related to misfolded PrP may occur in AD. Hence, it is even considered that common therapies could be applied for these PMDs [54].

As illustrated schematically in Fig. 10, we postulate that Tau phosphorylation may be an important factor enhancing neurotoxicity of cytoPrP. It has been reported that in TSE misfolded PrP (i) induces elevated phosphorylation of Tau [28,29] and (ii) directly inhibits proteasome activity, thus facilitating accumulation of cytoPrP [9]. Both processes may lead to the disassembly of microtubular cytoskeleton, the first through a loss of microtubule stabilization by phospho-Tau and increased susceptibility of Tau-depleted tubulin to the PrP-induced oligomerization/aggregation, and the second — by allowing cytosolic interaction of PrP with tubulin. It is plausible that even at low concentrations of cytoPrP, a synergistic effect of Tau hyperphosphorylation may evoke substantial toxicity at the level of microtubular cytoskeleton of neurons. Taken together, our studies point to a novel link between pathogeneses related to Tau and PrP.

Supplementary materials related to this article can be found online at doi:10.1016/j.bbamcr.2011.06.016.

Acknowledgments

This study was supported by research grant NN303 470538 from the Ministry of Science and Higher Education to K.N. and by statutory funds from the Nencki Institute of Experimental Biology. K.M.O. was partially supported by a Mazovian fellowship for Ph.D. students. We are grateful to Prof. Witold Surewicz for the plasmid encoding PrP and Prof. Peter S. Klein for the plasmid encoding Tau (Addgene plasmid 16316). Microscopic studies were performed in the Laboratory of Electron Microscopy (Nencki Institute of Experimental Biology) using equipment installed within the project sponsored by the EU Structural Funds: Centre of Advanced Technology BIM - Equipment purchase for the Laboratory of Biological and Medical Imaging. The authors thank Rafal Bartosiewicz and Henryk Bilski for expert assistance at the electron microscopy facility.

References

[1] S.B. Prusiner, Prions, Proc Natl Acad Sci USA 95 (1998) 13363–13383.
[2] J.I. Kim, I. Cali, K. Surewicz, Q. Kong, G.J. Raymond, R. Atarashi, B. Race, L. Qing, P. Gambetti, B. Caughey, W.K. Surewicz, Mammalian prions generated from bacterially expressed prion protein in the absence of any mammalian cofactors, J Biol Chem 285 (2010) 14083–14087.
[3] N. Stahl, D.R. Borchelt, S.B. Prusiner, Differential release of cellular and scrapie prion proteins from cellular membranes by phosphatidylinositol-specific phospholipase C, Biochemistry 29 (1990) 5405–5412.
[4] R.S. Hegde, J.A. Mastrianni, M.R. Scott, K.A. DeFea, P. Tremblay, M. Torchia, S.J. DeArmond, S.B. Prusiner, V.R. Lingappa, A transmembrane form of the prion protein in neurodegenerative disease, Science 279 (1998) 827–834.
[5] S.J. Kim, R.S. Hegde, Cotranslational partitioning of nascent prion protein into multiple populations at the translocation channel, Mol Biol Cell 13 (2002) 3775–3786.
[6] G. Zanusso, R.B. Petersen, T. Jin, Y. Jing, R. Kanoush, S. Ferrari, P. Gambetti, N. Singh, Proteasomal degradation and N-terminal protease resistance of the codon 145 mutant prion protein, J Biol Chem 274 (1999) 23396–23404.
[7] J. Heske, U. Heller, K.F. Winklhofer, J. Tatzelt, The C-terminal globular domain of the prion protein is necessary and sufficient for import into the endoplasmic reticulum, J Biol Chem 279 (2004) 5435–5443.
[8] N.S. Rane, S.W. Kang, O. Chakrabarti, L. Feigenbaum, R.S. Hegde, Reduced translocation of nascent prion protein during ER stress contributes to neurodegeneration, Dev Cell 15 (2008) 359–370.
[9] M. Kristiansen, P. Deriziotis, D.E. Dimcheff, G.S. Jackson, H. Ovaa, H. Naumann, A.R. Clarke, F.W. van Leeuwen, V. Menéndez-Benito, N.P. Dantuma, J.L. Portis, J. Collinge, S.J. Tabrizi, Disease-associated prion protein oligomers inhibit the 26S proteasome, Mol Cell 26 (2007) 175–188.
[10] M. Miesbauer, A.S. Rambold, K.F. Winklhofer, J. Tatzelt, Targeting of the prion protein to the cytosol: mechanisms and consequences, Curr Issues Mol Biol 12 (2009) 109–118.
[11] J. Ma, R. Wollmann, S. Lindquist, Neurotoxicity and neurodegeneration when PrP accumulates in the cytosol, Science 298 (2002) 1781–1785.
[12] C. Grenier, C. Bissonnette, L. Volkov, X. Roucou, Molecular morphology and toxicity of cytoplasmic prion protein aggregates in neuronal and non-neuronal cells, J Neurochem 97 (2006) 1456–1466.
[13] K. Nieznanski, Interactions of prion protein with intracellular proteins: so many partners and no consequences? Cell Mol Neurobiol 30 (2010) 653–666.
[14] A.S. Rambold, M. Miesbauer, D. Rapaport, T. Bartke, M. Baier, K.F. Winklhofer, J. Tatzelt, Association of Bcl-2 with misfolded prion protein is linked to the toxic potential of cytosolic PrP, Mol Biol Cell 17 (2006) 3356–3368.
[15] O. Chakrabarti, R.S. Hegde, Functional depletion of mahogunin by cytosolically exposed prion protein contributes to neurodegeneration, Cell 137 (2009) 1136–1147.
[16] K. Nieznanski, H. Nieznanska, K.J. Skowronek, K.M. Osiecka, D. Stepkowski, Direct interaction between prion protein and tubulin, Biochem Biophys Res Commun 334 (2005) 403–411.
[17] K. Nieznanski, Z.A. Podlubnaya, H. Nieznanska, Prion protein inhibits microtubule assembly by inducing tubulin oligomerization, Biochem Biophys Res Commun 349 (2006) 391–399.
[18] K.M. Osiecka, H. Nieznanska, K.J. Skowronek, J. Karolczak, G. Schneider, K. Nieznanski, Prion protein region 23–32 interacts with tubulin and inhibits microtubule assembly, Proteins 77 (2009) 279–296.
[19] C.F. Dong, S. Shi, X.F. Wang, R. An, P. Li, J.M. Chen, X. Wang, G.R. Wang, B. Shan, B.Y. Zhang, J. Han, X.P. Dong, The N-terminus of PrP is responsible for interacting with tubulin and fCJD related PrP mutants possess stronger inhibitive effect on microtubule assembly in vitro, Arch Biochem Biophys 470 (2008) 83–92.
[20] E.G. Gray, Spongiform encephalopathy: a neurocytologist's viewpoint with a note on Alzheimer's disease, Neuropathol Appl Neurobiol 12 (1986) 149–172.
[21] E. Nogales, Structural insights into microtubule function, Annu Rev Biochem 69 (2000) 277–302.
[22] L.A. Amos, D. Schlieper, Microtubules and maps, Adv Protein Chem 71 (2005) 257–298.
[23] J. Avila, J.J. Lucas, M. Perez, F. Hernandez, Role of tau protein in both physiological and pathological conditions, Physiol Rev 84 (2004) 361–384.
[24] B. Ghetti, F. Tagliavini, C.L. Masters, K. Beyreuther, G. Giaccone, L. Verga, M.R. Farlow, P.M. Conneally, S.R. Dlouhy, B. Azzarelli, O. Bugiani, Gerstmann–Sträussler–Scheinker disease. II. Neurofibrillary tangles and plaques with PrP-amyloid coexist in an affected family, Neurology 39 (1989) 1453–1461.
[25] F. Tagliavini, G. Giaccone, F. Prelli, L. Verga, M. Porro, J.Q. Trojanowski, M.R. Farlow, B. Frangione, B. Ghetti, O. Bugiani, A68 is a component of paired helical filaments of Gerstmann–Sträussler–Scheinker disease, Indiana kindred, Brain Res 616 (1993) 325–329.
[26] B. Sikorska, P.P. Liberski, T. Sobów, H. Budka, J.W. Ironside, Ultrastructural study of florid plaques in variant Creutzfeldt–Jakob disease: a comparison with amyloid plaques in kuru, sporadic Creutzfeldt–Jakob disease and Gerstmann–Sträussler–Scheinker disease, Neuropathol Appl Neurobiol 35 (2009) 46–59.
[27] L. Reiniger, A. Lukic, J. Linehan, P. Rudge, J. Collinge, S. Mead, S. Brandner, Tau, prions and Aβ: the triad of neurodegeneration, Acta Neuropathol 121 (2011) 5–20.
[28] M. Pérez, A.I. Rojo, F. Wandosell, J. Díaz-Nido, J. Avila, Prion peptide induces neuronal cell death through a pathway involving glycogen synthase kinase 3, Biochem J 372 (2003) 129–136.
[29] J.P. Lopes, C.R. Oliveira, P. Agostinho, Role of cyclin-dependent kinase 5 in the neurodegenerative process triggered by amyloid-Beta and prion peptides: implications for Alzheimer's disease and prion-related encephalopathies, Cell Mol Neurobiol 27 (2007) 943–957.
[30] J. Han, J. Zhang, H. Yao, X. Wang, F. Li, L. Chen, C. Gao, J. Gao, K. Nie, W. Zhou, X. Dong, Study on interaction between microtubule associated protein tau and prion protein, Sci China C Life Sci 49 (2006) 473–479.
[31] M. Morillas, W. Swietnicki, P. Gambetti, W.K. Surewicz, Membrane environment alters the conformational structure of the recombinant human prion protein, J Biol Chem 274 (1999) 36859–36865.

[32] R. Zahn, C. von Schroetter, K. Wuthrich, Human prion proteins expressed in *Escherichia coli* and purified by high-affinity column refolding, FEBS Lett 417 (1997) 400–404.
[33] E.M. Mandelkow, M. Herrmann, U. Ruhl, Tubulin domains probed by limited proteolysis and subunit-specific antibodies, J Mol Biol 185 (1985) 311–327.
[34] M. Castoldi, A.V. Popov, Purification of brain tubulin through two cycles of polymerization-depolymerization in a high-molarity buffer, Protein Expr Purif 32 (2003) 83–88.
[35] D.B. Murphy, K.A. Johnson, G.G. Borisy, Role of tubulin-associated proteins in microtubule nucleation and elongation, J Mol Biol 117 (1977) 33–52.
[36] A. Fellous, J. Francon, A.M. Lennon, J. Nunez, Microtubule assembly in vitro. Purification of assembly-promoting factors, Eur J Biochem 78 (1977) 167–174.
[37] G. Lindwall, R.D. Cole, The purification of tau protein and the occurrence of two phosphorylation states of tau in brain, J Biol Chem 259 (1984) 12241–12245.
[38] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[39] C.M. Hedgepeth, L.J. Conrad, J. Zhang, H.C. Huang, V.M. Lee, P.S. Klein, Activation of the Wnt signaling pathway: a molecular mechanism for lithium action, Dev Biol 185 (1997) 82–91.
[40] T.J. Singh, T. Zaidi, I. Grundke-Iqbal, K. Iqbal, Modulation of GSK-3-catalyzed phosphorylation of microtubule-associated protein tau by non-proline-dependent protein kinases, FEBS Lett 358 (1995) 4–8.
[41] A. Sengupta, J. Kabat, M. Novak, Q. Wu, I. Grundke-Iqbal, K. Iqbal, Phosphorylation of tau at both Thr 231 and Ser 262 is required for maximal inhibition of its binding to microtubules, Arch Biochem Biophys 357 (1998) 299–309.
[42] J. Lefèvre, K.G. Chernov, V. Joshi, S. Delga, F. Toma, D. Pastré, P.A. Curmi, P. Savarin, The C terminus of tubulin, a versatile partner for cationic molecules: binding of Tau, polyamines, and calcium, J Biol Chem 286 (2011) 3065–3078.
[43] W.D. Howard, S.N. Timasheff, GDP state of tubulin: stabilization of double rings, Biochemistry 25 (1986) 8292–8300.
[44] E. Nogales, H.W. Wang, H. Niederstrasser, Tubulin rings: which way do they curve? Curr Opin Struct Biol 13 (2003) 256–261.
[45] P. Lundberg, M. Magzoub, M. Lindberg, M. Hällbrink, J. Jarvet, L.E. Eriksson, U. Langel, A. Gräslund, Cell membrane translocation of the N-terminal (1–28) part of the prion protein, Biochem Biophys Res Commun 299 (2002) 85–90.
[46] X.L. Li, G.R. Wang, Y.Y. Jing, M.M. Pan, C.F. Dong, R.M. Zhou, Z.Y. Wang, Q. Shi, C. Gao, X.P. Dong, Cytosolic PrP induces apoptosis of cell by disrupting microtubule assembly, J Mol Neurosci 43 (2011) 316–325.
[47] L. Serrano, A. Valencia, R. Caballero, J. Avila, Localization of the high affinity calcium-binding site on tubulin molecule, J Biol Chem 261 (1986) 7076–7081.
[48] R.A. Keates, Traces of brain microtubule-associated proteins affect dynamic properties of microtubules, Biochem Cell Biol 68 (1990) 1202–1209.
[49] K. Ishizawa, T. Komori, T. Shimazu, T. Yamamoto, T. Kitamoto, K. Shimazu, T. Hirose, Hyperphosphorylated tau deposition parallels prion protein burden in a case of Gerstmann–Sträussler–Scheinker syndrome P102L mutation complicated with dementia, Acta Neuropathol 104 (2002) 342–350.
[50] G. Giaccone, M. Mangieri, R. Capobianco, L. Limido, J.J. Hauw, S. Haïk, P. Fociani, O. Bugiani, F. Tagliavini, Tauopathy in human and experimental variant Creutzfeldt–Jakob disease, Neurobiol Aging 29 (2008) 1864–1873.
[51] N. Bons, N. Mestre-Frances, P. Belli, F. Cathala, D.C. Gajdusek, P. Brown, Natural and experimental oral infection of nonhuman primates by bovine spongiform encephalopathy agents, Proc Natl Acad Sci USA 96 (1999) 4046–4051.
[52] M.J. Bautista, J. Gutiérrez, F.J. Salguero, M.M. Fernández de Marco, J.L. Romero-Trevejo, J.C. Gómez-Villamandos, BSE infection in bovine PrP transgenic mice leads to hyperphosphorylation of tau-protein, Vet Microbiol 115 (2006) 293–301.
[53] G. Leuba, K. Saini, A. Savioz, Y. Charnay, Early-onset familial Alzheimer disease with coexisting beta-amyloid and prion pathology, JAMA 283 (2000) 1689–1691.
[54] R. Morales, L.D. Estrada, R. Diaz-Espinoza, D. Morales-Scheihing, M.C. Jara, J. Castilla, C. Soto, Molecular cross talk between misfolded proteins in animal models of Alzheimer's and prion diseases, J Neurosci 30 (2010) 4528–4535.